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WHAT IS CLAIMED IS:

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- 1 1. An improved method of cloning a viable animal by nuclear  
2 transfer comprising the steps of:
  - 3 (a) inserting a NENS somatic cell, or nucleus isolated from  
4 said somatic cell, deriving from a somatic cell culture having undergone 5 or more  
5 passages, into an enucleated oocyte to form a cybrid;
  - 6 (b) activating the cybrid;
  - 7 (c) culturing the activated cybrid;
  - 8 (d) transferring the activated cybrid of step (c) into an  
9 appropriate host such that the activated cybrid develops into a fetus;
  - 10 (e) maintaining the fetus in the host until the fetus is capable of  
11 surviving and maturing into a viable animal outside of said host.
- 1 2. An animal made by the method of claim 1.
- 1 3. An organ or tissue made by the method of claim 1.
- 1 4. An embryo made by the method of claim 1.
- 1 5. A fetus made by the method of claim 1.
- 1 6. A cell line derived from cells made by the method of claim 1.

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1           7.     An improved method of cloning a mammal by nuclear  
2 transfer comprising the introduction of a donor cell from the mammal, or donor  
3 cell nucleus, into an enucleated oocyte of the same species as the donor cell to  
4 form a cybrid, inserting the cybrid into the uterus of a host mother of said species  
5 so as to cause implantation of the cybrid into the uterus to form a fetus, and  
6 permitting the fetus to develop into the cloned mammal wherein the improvement  
7 comprises using as the donor cell, or donor cell nucleus, a NENS somatic cell that  
8 has been cultured for more than five (5) passages.

1           8.     An animal made by the method of claim 7.

1           9.     An organ or tissue made by the method of claim 7.

1           10.    An embryo made by the method of claim 7.

1           11.    A fetus made by the method of claim 7.

1           12.    A cell line derived from cells made by the method of  
2 claim 7.

1           13.    A method for cloning an animal, said method comprising  
2 the steps of:

3           (a)     obtaining NENS somatic cells;

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- 4 (b) culturing said NENS somatic cells for 5 or more passages;  
5 (c) inserting the cultured NENS somatic cells of step (b), or  
6 nucleus isolated from said cultured NENS somatic cell, into an enucleate oocyte  
7 to form a cybrid;  
8 (d) activating the cybrid;  
9 (e) culturing the activated cybrid;  
10 (f) transferring the activated cybrid of step (e) into an  
11 appropriate host such that the activated cybrid develops into a fetus;  
12 (g) maintaining the fetus in the host until said fetus is capable  
13 of surviving as a viable animal outside of said host.

1 14. An animal made by the method of claim 1.

1 15. An organ or tissue made by the method of claim 1.

1 16. An embryo made by the method of claim 1.

1 17. A fetus made by the method of claim 1.

1 18. A cell line derived from cells made by the method of  
2 claim 1

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1           19.    A method for cloning a mammal with a cloning efficiency  
2 of better than ten percent (10%), said method comprising the steps of:

3           (a)    inserting a somatic cell, or nucleus isolated from said  
4 somatic cell, deriving from a somatic cell culture having undergone 5 or more  
5 passages, into an enucleate oocyte to form a cybrid;

6           (b)    activating the cybrid;

7           (c)    culturing the activated cybrid;

8           (d)    transferring the activated cybrid of step (c) into an  
9 appropriate host such that the activated cybrid develops into a fetus;

10          (e)    maintaining the fetus in the host until the fetus is capable of  
11 surviving and maturing into a viable animal outside of said host;

12 wherein the cloning efficiency of such method is better than ten percent (10%).

1           20.    A method for the cloning of a male mammal, said method  
2 comprising the steps of:

3           (a)    inserting a male somatic cell, or nucleus isolated from said  
4 somatic cell, deriving from a somatic cell culture having undergone 5 or more  
5 passages, into an enucleate oocyte to form a cybrid;

6           (b)    activating the cybrid;

7           (c)    culturing the activated cybrid;

8           (d)    transferring the activated cybrid of step (c) into an  
9 appropriate host such that the activated cybrid develops into a fetus;

10          (e)    maintaining the fetus in the host until the fetus is capable of  
11 surviving as a viable animal outside of said host.

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1                   21.    The method of claim 20 wherein the male somatic cell is a  
2 male NENS somatic cell.

1                   22.    The method of claim 20 wherein the said male somatic cell  
2 derives from a somatic cell culture having undergone 10 or more passages.

1                   23.    A method for improving blastocyst development rates from  
2 cybrids produced by nuclear transfer from a donor cell to an enucleated oocyte,  
3 said method comprising the steps of:

4                   (a)    activating the enucleated oocyte with an inhibitor selected  
5 from the group consisting of: protein kinase inhibitor and a protein synthesis  
6 inhibitor, prior to, during or after fusion with the donor cell nucleus; and

7                   (b)    electrostimulating the cybrid prior to, during or after fusion.

1                   24.    A method for producing an animal clone with targeted  
2 genetically-engineered targeted genetic alterations, said method comprising the  
3 steps of:

4                   (a)    altering in a targeted manner the nuclear DNA of somatic  
5 cells to produce genetically-altered cells;

6                   (b)    culturing the somatic cells of step (a) for five (5) or more  
7 passages to allow selection for the genetically-altered cells;

8                   (c)    inserting the altered nuclear DNA of the somatic cells of  
9 step (b) into an enucleate oocyte to form a cybrid;

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- 10 (d) activating the cybrid;  
11 (e) culturing the activated cybrid to form an embryo;  
12 (f) transferring the embryo into an appropriate host such that  
13 the embryo develops into a fetus;  
14 (g) maintaining said fetus in said host until said fetus is capable  
15 of surviving and maturing into a viable animal outside of said host.

1 25. An animal made by the method of claim 1.

1 26. An organ or tissue made by the method of claim 1.

1 27. An embryo made by the method of claim 1.

1 28. A fetus made by the method of claim 1.

1 29. A cell line derived from cells made by the method of  
2 claim 1.

1 30. An improved method of cloning a mammal by nuclear  
2 transfer comprising:

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3 (a) the introduction of a donor cell from the mammal, or donor  
4 cell nucleus, into an enucleated oocyte of the same species as the donor cell to  
5 form a cybrid,;

6 (b) inserting the cybrid into the uterus of a host mother of said  
7 species so as to cause implantation of the cybrid into the uterus to form a fetus,  
8 and permitting the fetus to develop into the cloned mammal

9 wherein the improvement comprises using as the donor cell, or donor cell nucleus,  
10 a somatic cell that has been cultured for more than five (5) passages, and wherein  
11 the donor cell, or donor cell nucleus, has been genetically transformed to comprise  
12 at least one addition, substitution or deletion of a nucleic acid or nucleic acid  
13 sequence.

1 31. An animal made by the method of claim 30.

1 32. An organ or tissue made by the method of claim 30.

1 33. An embryo made by the method of claim 30.

1 34. A fetus made by the method of claim 30.

1 35. A cell line derived from cells made by the method of  
2 claim 30.

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1 36. A process by which genetically-altered and genetically-non-  
2 altered animals may be produced, such process comprising the steps of:

3 (a) isolating a diploid donor cell;

4 (b) culturing the diploid donor cell for more than about 20 cell  
5 doublings;

6 (c) optionally altering in a targeted manner the genome of one  
7 or more cells of the diploid donor cells of step (b);

8 (d) optionally screening and selecting from the cells of step (c)  
9 stable desired mutants;

10 (e) reconstituting an embryo employing nuclei transfer  
11 techniques using nuclei from the cells of step (b), or optionally steps (c) or (d);

12 (f) culturing the embryo *in vivo* or *in vitro* to a blastocyst;

13 (g) optionally screening and selecting from the blastocysts of  
14 step (f) stable desired mutants;

15 (h) transfer of the blastocysts of steps (f) or (g) to medium  
16 capable of allowing the blastocyst to develop into a term animal.

1 37. An animal made by the method of claim 36.

1 38. An organ or tissue made by the method of claim 36.

1 39. An embryo made by the method of claim 36.

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1 40. A fetus made by the method of claim 36.

1 41. A cell line derived from cells made by the method of  
2 claim 36.

1 42. An improved method for cloning a term animal, said  
2 method comprising the steps of:

3 (a) inserting a somatic cell, or nucleus isolated from said  
4 somatic cell, deriving from a somatic cell culture having undergone 5 or more  
5 passages, into an enucleate oocyte to form a cybrid;

6 (b) optionally activating the cybrid;

7 (c) culturing the cybrid;

8 (d) transferring the cybrid of step (c) into an appropriate host  
9 such that the cybrid develops into a fetus;

10 (e) maintaining the fetus in the host until the fetus is capable of  
11 surviving and maturing into a term animal outside of said host.

1 43. An animal made by the method of claim 42.

1 44. An organ or tissue made by the method of claim 42.

1 45. An embryo made by the method of claim 42.

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46. A fetus made by the method of claim 42.

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47. A cell line derived from cells made by the method of

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claim 42.

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